Biosynthesis of Silver Nanoparticles (AgNPs) using waste fruits peel as an antimicrobial drug agent

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INTRODUCTION

Buzzing of nanotechnology in each and every aspect of science and technology has been booming at a tremendous rate now a day. Started its journey from organic chemistry, this field has now even reached to aeronautical research, and a special attention has been drawn in the medical and allied branches for exploitation of the nanotech for attending the limitations of the current scenario. Carrying foreword the success of nanotechnology in field of physical, chemical and medical sciences, it has now started revolutionizing the drug delivery sciences (Jain *et al.,* 2011).

Concept of Nanotechnology-:

Nanotechnology (sometimes shortened to "**nanotech**") is the study of manipulating matter on an atomic and molecular scale. Generally nanotechnology deals with structures sized between 1 to 100 nanometer in at least one dimension, and involves developing materials or devices within that size. Quantum mechanical effects are very important at this scale.

One nanometer (nm) is one billionth, or 10^{-9}, of a meter. By comparison, typical carbon-carbon bond lengths, or the spacing between these atoms in a molecule, are in the range 0.12–0.15 nm, and a DNA double-helix has a diameter around 2 nm. On the other hand, the smallest cellular life-forms, the bacteria of the genus Mycoplasma, are around 200 nm in length. To put that scale in another context, the comparative size of a nanometer to a meter is the same as that of a marble to the size of the earth (Kahn, 2006).

Nanoparticles possess unique electrical, optical as well as biological properties and are thus applied in catalysis, biosensing, imaging, drug delivery, nanodevice fabrication and in medicine (Nair and Laurencin, 2007). In the past few years, there has been an increasing interest in silver nanoparticles on account of the antimicrobial properties that they display (Choi *et al.,* 2008). They are even being projected as future generation antimicrobial agents (Rai *et al.,* 2009).

Two main approaches are used in nanotechnology. In the "bottom-up" approach, materials and devices are built from molecular components which assemble themselves chemically by principles of molecular recognition. In the "top-down" approach, nano-objects are constructed from larger entities without atomic-level control (Rodgers, 2006).

History of Nanotechnology -:

The term "nanotechnology" was defined by Tokyo Science University Professor Norio Taniguchi in a 1974 paper (Taniguchi, 1974) as follows: "'Nano-technology' mainly consists of the processing of, separation, consolidation, and deformation of materials by one atom or by one molecule."

In the 1980s the basic idea of this definition was explored in much more depth by Dr. K. Eric Drexler, who promoted the technological significance of nano-scale phenomena and devices through speeches and the books Engines of Creation: The Coming Era of Nanotechnology (1986) and *Nanosystems: Molecular Machinery, Manufacturing, and Computation* (Drexler, 1991), and so the term acquired its current sense.

Nanotechnology and nanoscience got started in the early 1980s with two major developments; the birth of cluster science and the invention of the scanning tunneling microscope (STM). In another development, the

synthesis and properties of semiconductor nanocrystals was studied; this led to a fast increasing number of metal and metal oxide nanoparticles and quantum dots.

In 2000, the United States National Nanotechnology Initiative was founded to coordinate Federal nanotechnology research and development and is evaluated by the President's Council of Advisors on Science and Technology.

Principle of biosynthesis of Nanoparticles -:

The production of metal-based nanoparticles by chemical reduction (Peterson *et al.*, 2007), thermal treatment (Sun and Luo, 2005), irradiation (Shao and Yao, 2006) and laser ablation (Tsuji *et al.*, 2002) often times requires the use of organic solvents and toxic reducing agents like sodium borohydride and N,N-dimethylformamide. Therefore, biological and biomimetic approaches for the synthesis of nanomaterials are being explored. Cell mass or extracellular components from microorganisms, such as *Klebsiella pneumoniae*, *Bacillus licheniformis*, *Fusarium oxysporum*, *Aspergillus flavus*, *Cladosporium cladosporioides*, *Aspergillus clavatus*, and *Penicillium brevicompactum* (Ahmad *et al.*, 2003; Shahverdi *et al.*, 2007; Kalishwaralal *et al.*, 2008; Balaji *et al.*, 2009; Shaligram *et al.*, 2009; Verma *et al.*, 2010) have been utilized for the reduction of silver ions to AgNPs. The unexploited plant resources for the synthesis of silver nanoparticles, various plant leaf extracts such as *Helianthus annus*, *Basella alba*, *Oryza sativa*, *Saccharum officinarum*, *Sorghum bicolour* and *Zea mays*; *Capsicum annuum* L.; *Pelargonium graveolens*; *Carica papaya*; *Chenopodium album*; *Rosa rugosa*; *Jatropha curcas*; *Aloe vera*; *boswellia* ovalifoliolata (Leela and Vivekanandan 2008; Shikuo *et al.*, 2007; Shankar *et al.*, 2008; Mude *et al.*, 2008; Dwivedi and Gopal 2010; Dubey *et al.*, 2010; Bar *et al.*, 2009; Chandran *et al.*, 2008; Ankanna *et al.*, 2010).

Development of reliable and eco-friendly process for synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology. One of the options to achieve this objective is to use natural processes such as use of biological systems. One approach that shows immense potential is based on the biosynthesis of nanoparticles using biological waste plant products such as fruit peel.

The principle of preparation of silver nanoparticles by using microorganism is a bioreduction process; the silver ions are reduced by the extracellular reductase enzymes produced by the microorganisms to silver metal in nanometer range. The reaction involved in this process is shown in Fig-I.

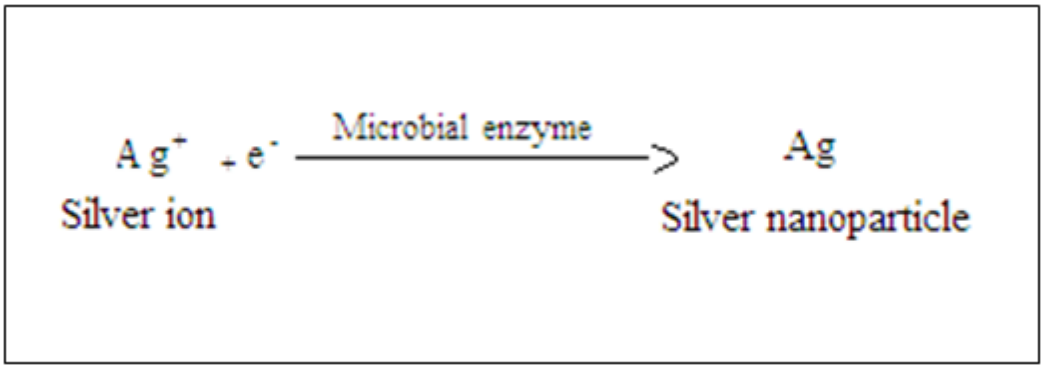

Fig-I: Reaction showing preparation of silver nanoparticles.

It is concluded from protein assay of microorganisms that the reductase involved in bioreduction for preparation of silver nanoparticles is an NADH-dependent reductase. The enzyme reductase gains electrons from NADH and oxidizes it to NAD+. The enzyme is then oxidized by the simultaneous reduction of Silver ions forming silver metal in nano form. In some cases a nitrate-dependent reductase is responsible for the bioreduction process, whereas, in case of rapid extracellular synthesis of nanoparticles the reduction happens within few minutes, therefore, a complex electron shuttle materials may be involved in the biosynthesis process (Moghaddam, 2010).

The ability to tune the optical absorption emission properties of semiconductor nano-particles (the so called quantum dots) by simple variation in nanoparticles size is particularly attractive in the facile band gap engineering of materials and the growth of quantum dots lasers. More recently nanoscale matter has been locked at with interest for potential applications in nanocomputers. Synthesis of advanced materials energy storage devices, electronic and optical displays chemical and biosensors as well as biomedical devices, recognizing the importance of nanomaterials in key future technology.

Characteristics of Plants Waste Product taken -:

According to the literature survey, many reports were present on biosynthesis of silver nanoparticles from plant extracts but only few reports were present on the biosynthesis of silver nanoparticles from waste plant products. In this study we will take fruit peel of three different plants (*Citrus limetta, Punica granatum* and *Pisum Sativum*).

1) Citrus limetta Risso. *-:*

CLASSIFICATION	
GROUP	Dicotyledons
CLASS	Polypetalae
SERIES	Disciflorae
ORDER	Geraniales
FAMILY	Rutaceae
GENUS	*Citrus*
SPECIES	*limetta*

Citrus limetta is a species of citrus. Common names for varieties of this species include sweet limetta, Mediterranean sweet lemon, sweet lemon, and sweet lime. It is a small tree which may reach 8m in height. The sweet lemon has irregular branches, and relatively smooth, brownish-grey bark. It possesses numerous thorns which may grow to anywhere from 1.5 to 7.5 cm long. The petioles of the sweet lemon are narrowly but distinctly winged, and are 8 to 29 mm long. It has leaflet rather than leaves, which are obovate and 5.5 to 17 cm wide. The apex of the leaflet is acuminate, and the base of the leaflet is rounded. Flowers are white in bud and in bloom, ranging from 2 to 3 cm wide. The petals soon fall away, leaving the fruit to grow. The skin of the fruit is light yellow at maturity; the rind is white and about 5 mm thick. The pulp is greenish and the juice is sweet rather than acidic.

The fruit of the sweet lemon is edible, and contains essential oils. The tree is used for ornamental purposes or for graft stock. In Iran, it is particularly used to treat flu and cold. The fruit has high levels of ascorbic acid (Vitamin C). The juice is used by all, especially those who are sick.

2) *Punica granatum* L. -:

CLASSIFICATION	
GROUP	Dicotyledons
CLASS	Polypetalae
SERIES	Calyciflorae
ORDER	Myrtales
FAMILY	Lythraceae
GENUS	*Punica*
SPECIES	*granatum*

Punica granatum is a fruit-bearing deciduous shrub or small tree growing between five and eight meters tall. In the Indian subcontinent's ancient Ayurveda system of medicine, the pomegranate has extensively been used as a source of traditional remidies for thousands of years (Jindal and Sharma, 2004).

The rind of the fruit and the bark of the pomegranate tree is used as a traditional remedy against diarrhea, dysentery and intestinal parasites (Jindal and Sharma, 2004). Pomegranate juice (of specific fruit strains) is also used as eyedrops as it is believed to slow the development of cataracts (Lad, 2002).

Pomegranate aril juice provides about 16% of an adult's daily vitamin C requirement per 100 ml serving, and is a good source of vitamin B_5 (Pantothenic acid), potassium and polyphenols, such as tannins and flavinoids (Schubert *et al.*, 1999). Other phytochemicals include polyphenolic catechins, gallocatechins, and anthocyanins, such as prodelphinidins, delphinidin, cyaniding and pelargonidin (Plumb *et al.*, 2002). Juice of the pomegranate may be effective in reducing heart disease risk factors, including LDL oxidation, macrophage oxidative status, and foam cell formation (Esmailzadeh *et al.*, 2004).

3) Pisum sativum L.-:

CLASSIFICATION	
GROUP	Dicotyledons
CLASS	Polypetalae
SERIES	Calyciflorae
ORDER	Rosales
FAMILY	Leguminosae
GENUS	*Pisum*
SPECIES	*sativum*

Pisum sativum is an annual plant, with a life cycle of one year. It is a cool season crop grown in many parts of the world; planting can take place from winter to early summer depending on location. The average pea

weighs between 0.1 and 0.36 gms. The species is used as a vegetable, fresh, frozen or canned, and is grown to produce dry peas like the split pea.

The peels extract of *Pisum sativum* act as antioxidative potential (Dixit and Kar, 2009). Pea is high in fiber, protein, vitamins, minerals and lutein. Dry weight is about one-quarter protein and one-quarter sugar. Pea seed peptide fractions have less ability to scavenge free radicals than glutathione, but greater ability to chelate metals and inhibit linoleic acid oxidation (Pownall *et al.*, 2010). Bioplastic can be made using pea starch.

Target Microbial Species -:

1) Staphylococcus aureus-:

Staphylococcus aureus is a facultative anaerobic, Gram-positive coccus and is the most common cause of staph infections. It is frequently part of the skin flora found in the nose and on skin (Kluytmans *et al*., 1997). The carotenoid pigment staphyloxanthin is responsible for S. aureus characteristic golden colour, which may be seen in colonies of the organism. This pigment acts as a virulence factor with an antioxidant action that helps the microbe evade death by reactive oxygen species used by the host immune system.

S. aureus can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections. *S. aureus* was discovered in Aberdeen, Scotland in 1880 by the surgeon Sir Alexander Ogston in pus from surgical abscesses (Ogston, 1984).

2) Bacillus fusiformis-:

Bacillus fusiformis is a long rod – shaped organism measuring 5-10 x 0 .6-0.8 u, slightly swollen in the middle and pointed at the ends. It stains readily with the ordinary dyes. The majority of observers state that it is gram positive. It is nearly always associated with the Borrelia vincentii.

This organism was described originally as occurring in cases of hospital gangrene. It is more common at present in the form of Vincent's angina, an inflammatory condition of the throat. It is known to be the casual agent of Vincent gingivitis. The chief symptoms are, swollen, bleeding gums, small, painful ulcers covering the gums and tooth margins and characteristic fetid breath. The ulcers may spread to the throat and tonsils.

3) Enterobacter aerogenes-:

Enterobacter aerogenes is a Gram negative, nosocomial and pathogenic bacterium that causes opportunistic infections including most types of infections. They quickly become resistant to standard antibiotics during treatment, requiring change in antibiotic to avoid worsening of the sepsis.

Various infections includes bacteremia, lower respiratory tract infections, skin and soft tissue infections, urinary tract infections, endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, and ophthalmic infections.

Some of the infections caused by *E. aerogenes* result from specific antibiotic treatments, venous catheter insertions and surgical procedures. *E. aerogenes* is generally found in the human gastrointestinal tract and does not generally cause disease in healthy individuals. It has been found to live in various wastes, hygienic chemicals and soil. The bacterium also has some commercial significance – the hydrogen gas produced during fermentation has been experimented with using molasses as the substrate.

4) Candida albicans-:

Candida albicans is encountered in all clinical forms of candidiasis. Other species exits as normal flora of the cutaneous and mucocutaneous areas, (Rippon, 1982) although they show a limited pathogenic potential. (Hopfer, 1985) *C. stellatoidea* and *C. tropicalis* have often been recovered from cases of vaginitis and nail involvement; C. *parapsilosis* and *C. guilliermondii* are associated with skin and nail infections.

Candida albicans is polymorphic and exhibits dimorphism (Smith, 1985). It grows in four distinct phases or forms, viz. blastoconidia or yeast, chlamydoconidia, pseudomycelium and true mycelium. The demonstration, isolation and identification of the yeast or mycelial forms of *Candida* is usually simple. However, the near ubiquitous nature of *C. albicans* as a commensal poses problems in interpretation of cultural and immunological findings. *Candida albicans* is a part of the normal microbiota of the gastrointestinal tract, mouth, and vaginal area causing systemic mycoses. In the absence of the normal microbiota the fungus can proliferate and causes disease involving the skin or mucous membranes.

5) *Aspergillus fumigatus-:*

Aspergillus fumigatus is a saprophytic fungus of the genus *Aspergillus*, and is one of the most common *Aspergillus* species to cause disease in immuno-compromised individuals. *A. fumigatus* is a saprotroph that is widespread in nature, typically found in soil and decaying organic matter such as compost heaps, where it plays an essential role in carbon and nitrogen recycling.

When the fermentation broth of *A. fumigatus* was screened, a number of indolic alkaloids with anti-mitotic properties were discovered (Cui *et al.*, 1996). The compounds of interest have been of a class known as tryprostatins, with spirotryprostatin B being of special interest as an anti-cancer drug. *A. fumigatus* grown on certain building materials can produce genotoxic and cytotoxic mycotoxins such as gliotoxin (Nieminen *et al.*, 2002).

6) Microsporum fulvum-:

Microsporum is a genus of fungi that causes tinea capitis, tinea corpus, ringworm, and other dermatophytoses. *Microsporum* forms both macroconidia and microconidia on short conidiophores. Macroconidia are hyaline, multiseptate, variable in form, fusiform, spindle-shaped to obovate, ranging from 7 to 20 by 30 to 160 µm in size. *Microsporum fulvum* colonies are fast growing, flat, suede-like, tawny-buff in colour and frequently have a fluffy white advancing edge.

Microsporum fulvum is a geophilic fungus of world-wide distribution which may cause occasional infections in humans and animals. It extends deeper into the epidermis as well invasive hair and nail diseases i.e. cutaneous mycosis. It causes a superficial dermatophytic infection characterized by either inflammatory or non inflammatory lesions on the glabrous skin (i.e. skin regions except the scalp, groin, palms and soles).

Antibiotics -:

An antibiotic is a compound or substance that kills or slows down the growth of microbes. However, with increased knowledge of the causative agents of various infectious diseases, antibiotic has come to denote a broader range of antimicrobial compounds, including antifungal and antibacterial compounds. The term antibiotic was coined by Selman Waksman in 1942 to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution (Waksman, 1947). With advances in medicinal chemistry, most of today's antibiotics are chemically semi synthetic modifications of various natural compounds.

Antimicrobial Pharmacodynamics

The successful outcome of antimicrobial therapy with antibiotic compounds depends on several factors. These include host defense mechanisms, the location of infection, and the pharmacokinetic and pharmacodynamic properties of the antibiotics (Pankey and Sabath, 2004). A bactericidal activity of antibacterial may depend on the bacterial growth phase, and it often requires ongoing metabolic activity and division of bacterial cells (Mascio *et al.,* 2007).

Antibiotic Resistance

The emergence of resistance of microbes to antibiotic drugs is a common phenomenon. Emergence of resistance often reflects evolutionary processes that take place during antibiotic drug therapy. The antibiotic treatment may select for microbial strains with physiologically or genetically enhanced capacity to survive high doses of antibiotics. Under certain conditions, it may result in preferential growth of resistant microbes, while growth of susceptible microbes is inhibited by the drug (Levy, 1994).

Survival of bacteria often results from an inheritable resistance (Witte, 2004). Resistance to antibacterial also occurs through horizontal gene transfer. Horizontal transfer is more likely to happen in locations of frequent antibiotic use. Antibacterial such as penicillin and erythromycin, which used to have high efficacy against many bacterial species and strains, have become less effective, because of increased resistance of many bacterial strains. Antibacterial resistance may impose a biological cost, thereby reducing fitness of resistant strains, which can limit the spread of antibacterial-resistant bacteria, for example, in the absence of antibacterial compounds. Additional mutations, however, may compensate for this fitness cost and can aid the survival of these microbes (Andersson, 2006).

Spherical silver nanoparticles showed potent activity against *Trichophyton mentagrophytes*, *Trichosporon beigelii* and *Candida albicans* compared with that of commercially available antifungal agent amphotericin B and fluconazole (Kim *et al.,* 2008). Stable colloidal solutions containing up to 35 ppm nanoparticles were found to have effective antifungal properties against *Aspergillus, Penicillium* and *Trichoderma* species (Petica *et al.,* 2008).

REVIEW OF LITERATURE

Production of silver nanoparticles using plant extract-:

Torresday *et al.*, (2002) reported the preparation and study of quantum dots and quantum wires play a very important role in nanotechnology. In this particular study, the report on the uptake of silver by living alfalfa plants. X-ray absorption spectroscopy and Transmission Electron Microscopy (TEM) studies corroborated silver metal uptake by alfalfa plants from a silver-rich solid medium and the subsequent formation of silver nanoparticles. Silver nanoparticle alignment, structure, and coalescence were observed using TEM with anatomic resolution analysis. Dark field image TEM showed the connection of silver nanoparticles of different sizes by possibly noncrystalline silver atomic wires.

Balaprasad *et al.*, (2005) reported biosynthesis of gold and silver Nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution. On treating aqueous silver sulfate and chloroauric acid solutions with *Emblica officinalis* fruit extract, rapid reduction of silver and chloroaurate ions is observed leading to the formation of highly stable silver and gold nanoparticles in solution. TEM analysis of the silver and gold nanoparticles indicated that they range in size from 10 to 20 nm and 15 to 25 nm respectively. Ag and Au nanoparticles thus synthesized were then phase transferred into an organic solution using a cationic surfactant octadecylamine. Transmetallation reaction between hydrophobized silver nanoparticles and hydrophobized chloroaurate ions in chloroform resulted in the formation of gold nanoparticles.

Li *et al.*, (2007) reported Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract. The results indicated that the proteins, which have amine groups, played a reducing and controlling role during the formation of silver NPs in the solutions, and that the secondary structure of the proteins changed after reaction with silver ions. The crystalline phase of the NPs changed from polycrystalline to single crystalline and increased in size with increasing reaction time.

Huang *et al.*, (2007) reported biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. Not only could silver nanoparticles ranging from 55 to 80 nm in size be fabricated, but also triangular or spherical shaped gold nanoparticles could be easily modulated by reacting the novel sundried biomass of *Cinnamomum camphora* leaf with aqueous silver or gold precursors at ambient temperature. The marked difference of shape control between gold and silver nanoparticles was attributed to the comparative advantage of protective biomolecules and reductive biomolecules. The polyol components and the water-soluble heterocyclic components were mainly responsible for the reduction of silver ions or chloroaurate ions and the stabilization of the nanoparticles, respectively. The sundried leaf in this work was very suitable for simple synthesis of nanoparticles.

Chandran *et al.*, (2008) reported synthesis of Gold Nanotriangles and Silver Nanoparticles using *Aloe vera* Plant Extract. Biogenic gold nanotriangles and spherical silver nanoparticles were synthesized by a simple procedure using *Aloe vera* leaf extract as the reducing agent. Reduction of silver ions by *Aloe vera* extract however, led to the formation of spherical silver nanoparticles of 15.2 nm ± 4.2 nm size.

Leela and Vivekanandan (2008) reported, tapping the unexploited plant resources for the synthesis of silver nanoparticles. The bioreduction behaviour of various plant leaf extracts such as *Helianthus annus* (*Asteraceae*), *Basella alba* (*Basellaceae*), *Oryza sativa*, *Saccharum officinarum*, *Sorghum bicolour* and *Zea mays* (*Poaceae*) in the synthesis of silver nanoparticles was investigated employing UV/Visible Spectrum (UV-Vis), X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM). *H. annus* was found to exhibit strong potential for rapid reduction of silver ions.

Shankar *et al.*, (2008) reported Geranium Leaf Assisted Biosynthesis of Silver Nanoparticles. On treating aqueous silver nitrate solution with geranium leaf extract, rapid reduction of the silver ions is observed leading to the formation of highly stable, crystalline silver nanoparticles in solution. The rate of reduction of the silver ions by the geranium leaf extract is faster than that observed by them in an earlier study using a fungus, *Fusarium oxysporum*. This study also represents an important advance in the use of plants over microorganisms in the biosynthesis of metal nanoparticles.

Mude *et al.*, (2008) reported the first report of synthesis of silver nanoparticles by using callus extract of *Carica papaya*. The formation of brown colour in the reaction mixture indicates the synthesis of silver nanoparticles. The further detection and characterization of these synthesized silver nanoparticles was carried by spectrophotometry. Fourier Transform Infrared Spectroscopy (FTIR) spectrum analysis showed peaks between 1000–2000 cm^{-1} which confirmed the presence of proteins and other ligands required for the synthesis and stabilization of silver nanoparticles. SEM micrograph confirmed the synthesis of spherical silver nanoparticles in the size range of 60–80 nm.

Bar *et al.*, (2009) reported green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*. An eco-friendly process for rapid synthesis of silver nanoparticles has been reported using aqueous seed extract of *Jatropha curcas*. Formation of stable silver nanoparticles at different concentration of $AgNO_3$ gives mostly spherical particles with diameter ranging from 15 to 50 nm. The resulting silver particles are characterized using TEM, XRD and UV–vis spectroscopic techniques.

Sinha *et al.*, (2009) reported nanoparticles fabrication using ambient biological resources. Nanotechnology has recently emerged as an elementary division of science and technology that investigates and regulates the interaction at cell level between synthetic and biological materials with the help of nanoparticles. Currently, simple prokaryotes to complex eukaryotic organisms including higher angiospermic plants are used for the fabrication of NPs. This article presents a review of the ambient biological systems that may support and revolutionize the art of fabrication of nanoparticles and the development of an updated knowledge base.

Shekhawat and Arya (2009) reported biological Synthesis of Ag Nanoparticles through *In Vitro* Cultures of *Brassica Juncea* C. zern. Seedlings of *B. juncea* prepared in vitro and 14-days old plants were transferred into nutrient solution augmented with silver nitrate (25-2000 μM) allow the plants to grow in hydroponic culture for seven days. Then, the plants were harvested and analyzed through UV-VIS and by TEM that confirms the nanoscale silver nanoparticles. We have found absorption peak in visible range (420-430 nm) of spectrum that is chiefly due to the silver nanoparticles. Moreover, size of the nanoparticles can also be controlled by altering some conditions like pH, concentration of $AgNO_3$ and temperature.

Krishnaraj *et al.*, (2009) reported **s**ynthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Silver nanoparticles were rapidly synthesized using leaf extract of *Acalypha indica* and the formation of nanoparticles was observed within 30 min. The results recorded from UV–vis spectrum, SEM, XRD and Energy Dispersive Spectroscopy (EDS) support the biosynthesis and characterization of silver nanoparticles. Further, the antibacterial activity of synthesized silver nanoparticles showed effective inhibitory activity against water borne pathogens Viz., *Escherichia coli* and *Vibrio cholerae*. Silver nanoparticles 10 μg/ml were recorded as the minimal inhibitory concentration (MIC) against *E. coli* and *V. cholerae*.

Jain *et al.*, (2009) reported synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. Nanoparticles were characterized using UV–Vis absorption spectroscopy, FTIR, XRD and SEM. XRD and SEM analysis showed the average particle size of 15 nm as well as revealed their cubic structure. Further these biologically synthesized nanoparticles were found to be highly

toxic against different multi drug resistant human pathogens (*Escherichia coli* and *Pseudomonas aeruginosa).* This is for the first time that any plant fruit extract was used for the synthesis of nanoparticles.

Jha *et al.*, (2009) reported biosynthesis of silver nanoparticles using *Eclipta* leaf. A green, low-cost and reproducible *Eclipta* leaves negotiated synthesis of silver nanoparticles is reported. The synthesis is performed at room temperature. X-ray and TEM analyses are performed to ascertain the formation of Ag nanoparticles. Nanoparticles almost spherical in shape having a size of 2–6 nm are found. UV-visible study revealed the surface plasmon resonance at 419 nm. The lattice strain is estimated to be 0.0045 using Williamson-Hall approach.

Raut *et al.*, (2010) reported extracellular synthesis of Silver Nanoparticles using dried leaves of *Pongamia pinnata* (L) Pierre. Stable and crystalline silver nanoparticles were formed by the treatment of aqueous solution of $AgNO_3$ (1mM) with dried leaf extract of *Pongamia pinnata* (L) Pierre. UV-visible spectroscopy studies were carried out to quantify the formation of silver nanoparticles. TEM, XRD and FTIR were used to characterize the silver nanoparticles. Water soluble heterocyclic compounds such as flavones were mainly responsible for the reduction and stabilization of the nanoparticles. Silver nanoparticles were effective against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Singhal *et al.*, (2010) reported biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. Biosynthesized nanoparticles were characterized with the help of UV–vis, Atomic Absorption Spectroscopy (AAS), Dynamic light scattering (DLS), XRD, FTIR, and TEM. Stability of bioreduced silver nanoparticles was analyzed using UV–vis absorption spectra, and their antimicrobial activity was screened against both *E. coli* and *S. aureus* microorganisms.

Dwivedi and Gopal (2010) reported biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. The aqueous leaf extract of the herb was used as mild reducing agent for silver and gold nanoparticles (SNPs and GNPs) synthesis from their salt solutions in single-pot process. Influence of leaf extract quantities, metal concentrations, contact time, reaction temperature and pH were evaluated to find their effects on NPs synthesis. The produced nanocrystals of silver and gold were analyzed with TEM, XRD, EDX and FTIR. The stability of NPs was evaluated at different pH with zeta potentiometer without adding any stabilizing agents.

Dubey *et al.*, (2010) reported green synthesis and characterizations of silver and gold nanoparticles using leaf extract of *Rosa rugosa*. Surface plasmon resonance spectra for silver and gold are obtained at 451 and 578 nm with brown yellow and pink-red color, respectively. AgNPs and AuNPs vary in size according to different leaves extract and metal concentration used for the synthesis. Different instrumental techniques were applied to characterize the synthesized AgNPs and AuNPs viz. UV–vis, TEM, XRD, FTIR, Zetasizer and EDX.

Ankanna *et al.*, (2010) reported production of biogenic silver nanoparticles using *Boswellia ovalifoliolata* stem bark. After exposing the silver ions to bark extract, rapid reduction of silver ions is observed leading to the formation of silver nanoparticles in solution. The most needed outcome of this work will be the development of value-added products from Boswellia *ovalifoliolata* for biomedical and nanotechnology based industries.

Farooqui *et al.*, (2010) describes the first report on the synthesis of silver nanoparticles using extracts of a medicinal leaf *Clerodendrum inerme*. Nanoparticles were synthesized from three different leaf conditions – fresh leaves, sun-dried leaves, and hot-air oven dried leaves. Atomic force microscopy (AFM) analysis of the nanoparticles revealed differences in sizes for the nanoparticles synthesized from different leaf conditions. Nanoparticles synthesized using fresh leaves possessed the smallest sizes. It is anticipated that optimization of the current synthesis method would yield highly mono-dispersed silver nanoparticles that have great potential in treating skin ailments.

Gilaki (2010) reported biosynthesis of Silver Nanoparticles using Plant Extracts. The bioreduction performance of different plant leaf remove such as *Helianthus annus* (Asteraceae), *Sorghum bicolour*, *Basella alba* (Basellaceae), *Oryza sativa*, *Saccharum officinarum* and *Zea mays* (Poaceae) in the synthesis of Ag nanoparticles was examined utilizing UV/Vis, XRD and SEM. *Helianthus annus* was found to exhibit strong potential for rapid reduction of Ag ions. It was observed that there is no association forever between the colour growth and the augment in absorbance displayed by the nanometal synthesized.

Rajani *et al.*, (2010) reported fabrication of biogenic Silver Nanoparticles using agricultural crop plant leaf extracts. Three pulse crop plants and three cereal crop plants (*Vigna radiata*, *Arachis hypogaea*, *Cyamopsis tetragonolobus*, *Zea mays*, *Pennisetum glaucum*, *Sorghum vulgare*) were used and compared for their extra cellular synthesis of metallic silver nanoparticles. Stable silver nanoparticles were formed by treating aqueous solution of $AgNO_3$ with the plant leaf extracts as reducing agent at temperatures 50 °C–95 °C. UV-Vis was utilized to monitor the formation of silver nanoparticles. XRD, SEM and EDAX analysis confirm the size of the formed silver nanoparticles. This could provide a faster synthesis rate comparable to those of chemical methods and potentially be used in areas such as cosmetics, food and medical applications.

Rajasekharreddy *et al.*, (2010) reported qualitative assessment of silver and gold nanoparticle synthesis in various plants: a photobiological approach. In this study, the extracellular production of Ag and Au nanoparticles was carried out from the leaves of the plants, *Tridax procumbens* L., *Jatropa curcas* L., *Calotropis gigantea* L., *Solanum melongena* L., *Datura metel* L., *Carica papaya* L. and *Citrus aurantium* L. by the sunlight exposure method. Among these *T. procumbens*, *J. curcas* and *C. gigantea* plants synthesized <20 nm sized and spherical-shaped Ag particles. The amount of nanoparticles synthesized and its qualitative characterization was done by UV–vis and TEM, respectively. XRD and XPS were used for structural confirmation. Further analysis carried out by FTIR, provided evidence for the presence of amino groups, which increased the stability of the synthesized nanoparticles.

Tripathy *et al.*, (2010) reported process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of *Azadirachta indica* leaves. The present study deals with investigating the effect of process variables like reductant concentrations, reaction pH, mixing ratio of the reactants and interaction time on the morphology and size of silver nanoparticles synthesized using aqueous extract of *Azadirachta indica* (Neem) leaves. By means of UV-VIS, XRD, SEM and TEM techniques, it was observed that the morphology and size of the nanoparticles were strongly dependent on the process parameters. Within 4 h interaction period, nanoparticles below 20-nm size with nearly spherical shape were produced. On increasing interaction time (ageing) to 66 days, both aggregation and shape anisotropy (ellipsoidal, polyhedral and capsular) of the particles increased.

Geethalakshmi and Sarada (2010) reported synthesis of plant-mediated silver nanoparticles using *Trianthema decandra* extract and evaluation of their anti microbial activities. Nanoparticles were characterized using UV–Vis absorption spectroscopy, FTIR, XRD and SEM. Further these biologically synthesized nanoparticles were found to be highly toxic against different multi drug resistant human pathogens. This is for the first time reporting that *Trianthema decandra* plant extract was used for the synthesis of nanoparticles.

Zhang *et al.*, (2010) reported synergetic antibacterial effects of silver nanoparticles *Aloe vera* prepared via a green method. Aloe Vera-conjugated Ag nanoparticles (AgNPs@AV hybrids) are synthesized in large quantities by reducing silver nitrate with Aloe Vera pulp extract at room temperature. TEM image reveals that these NPs are predominantly spherical with an average of 25 nm in diameter. The crystal structure of AgNPs@AV is determined by XRD. The cytotoxicity of AgNPs@AV hybrids is detected by carrying out the cell viability measurement on Human Dermal Fibroblasts (HDF) cells, the results show that no obvious cytotoxicity is observed. Compared with Vera gel and Ag NPs (washed from Vera gel) alone, AgNPs@AV hybrids possess more excellent antibacterial activity on *E. coli* even at very low concentration.

Saxena *et al.*, (2010) reported biological synthesis of silver nanoparticles by using onion (*allium cepa*) extract and their antibacterial activity. They have reported a fast, convenient and extracellular method for the synthesis of silver nanoparticles by reducing silver nitrate with the help of onion (*Allium cepa)* extract. The morphology of silver nanoparticles was confirmed by TEM. The antibacterial activity of these nanoparticles was studies against *E.coli* and *Salmonella typhimurium*. The bactericidal property of nanoparticles was analyzed by measuring the growth curve of bacteria and 50µg/ml concentration of silver nanoparticles was found to be effective antibacterial.

Elumalai *et al.*, (2010) reported green synthesis of silver nanoparticle using *Euphorbia hirta* L and their antifungal activities. Nanoparticles were characterized using UV-VIS absorption spectroscopy's. Green synthesized silver nanoparticles showed higher antifungal activity against *Candida albicans, C.kefyr, A.niger* whereas intermediated activity showed against *C.tropicalis, C.krusei, A.flavus, A.fumigatus*.

Vaseeharan *et al.*, (2010) reported antibacterial activity of silver nanoparticles (AgNPs) synthesized by tea leaf extracts against pathogenic *Vibrio harveyi* and its protective efficacy on juvenile *Feneropenaeus indicus*. AgNPs were synthesized by a simple procedure using tea leaf extract as the reducing agent. Bacteriological tests were performed in Luria-Bertani medium on solid agar plates and in liquid systems supplemented with *V. harveyi* against different concentrations of AgNPs. AgNPs synthesized in the present study were shown to be effective against *V. harveyi* isolated from *F. indicus*. The combined results of long and short-term treatment of AgNPs synthesized by tea leaf extract showed a 71% reduction in accumulated mortality. The AgNPs synthesized by tea leaf extract may be an alternative to antibiotics in controlling *V. harveyi* infections.

Prabhu *et al.*, (2010) reported synthesis of silver phyto nanoparticles and their antibacterial efficacy. The herbal leaves like *Ocimum sanctum* and *Vitex negundo* were included to analyze the productivity of nanoparticles. The silver phyto nanoparticles were collected from each herbal plant and tested their antibacterial activity. The test cultures included in this study were *Staphylococcus aureus, Vibrio cholerae, Proteus vulgaris and Pseudomonas aeruginosa.* The antibacterial activities of all the herbal nanoparticles obtained from *Ocimum sanctum* showed maximum inhibitory rate using 150µg of these plants extract compared with *Vitex negundo*. The silver nanoparticles from herbal leaves showed a good antibacterial activity than the plants used.

Khandelwal *et al.*, (2010) reported green synthesis of silver nanoparticles using *Argimone mexicana* leaf extract and evaluation of their antimicrobial activities. Nanoparticles were characterized using UV–Vis absorption spectroscopy, FTIR, XRD and SEM. Further these biologically synthesized nanoparticles were found to be highly toxic against different bacterial spp *(Aspergillus flavus, Escherichia coli* and *Pseudomonas aeruginosa)*. The most important outcome of this work will be the development of value-added products from *Argimone maxicana* (a potential weed of India) for biomedical and nanotechnology based industries.

Mahitha *et al.*, (2011) reported biosynthesis, characterization and antimicrobial studies of AgNPs extract from *Bacopa monniera* whole plant. UV-Vis spectrum, XRD, EDX evidences the presence of silver nanoparticles in the aquatic solution of leaf extract. FTIR analysis of the nanoparticles indicated the presence of proteins, which may be acting as capping agents around the nanoparticles. From TEM analysis, the size of the silver nanoparticles was measured (10-30 nm). Further the antimicrobial activity of synthesized particles showed effective inhibitory activity against S*taphylococcus aureus, Escherichia coli, Klebsiella pneumonia* and *Bacillus subtilis*.

Ahmad *et al.*, (2011) reported biosynthesis of Silver Nanoparticles from *Desmodium triflorum:* a novel approach towards weed utilization. UV-Vis spectrum, TEM and XRD showed the formation of well-dispersed silver nanoparticles. The process of reduction is extracellular and fast which may lead to the development of easy biosynthesis of silver nanoparticles. Plants during glycolysis produce a large amount of H^+ ions along with

NAD which acts as a strong redoxing agent; this seems to be responsible for the formation of AgNPs. Water-soluble antioxidative agents like ascorbic acids further seem to be responsible for the reduction of AgNPs. These AgNPs produced show good antimicrobial activity against common pathogens (*Staphylococcus* and *E.coli).*

To the best of my knowledge no report is available on the use of plant waste product in the synthesis of silver nanoparticles except peel of *Citrus sinensis*; *Musa paradisiaca* (Konwarh *et al.,* 2011; Bankar *et al.,* 2010).

Production of Silver Nanoparticles from waste fruit peel -:

Konwarh *et al.,* (2011) reported biomimetic preparation of polymer-supported free radical scavenging, cytocompatible and antimicrobial green silver nanoparticles using aqueous extract of *Citrus sinensis* peel. The compositional abundance of pectins, flavonoids, ascorbic acid, sugars, carotenoids and myriad other flavones may be envisaged for the effective reductive potential of orange peel to generate silver nanoparticles. The nanoparticles were distributed within a narrow size spectrum of (3-12 nm) with characteristic Bragg's reflection planes of fcc structure, and surface Plasmon resonance peak at 404 nm. Their synergy with rifampicin against *Bacillus subtilis* and cytocompatibility with the human leukemic monocytic cell line, THP-1 were also investigated.

Bankar *et al.,* (2010) reported banana peel extract mediated novel route for the synthesis of silver nanoparticles. Bio-inspired silver nanoparticles were synthesized with the aid of a novel, non-toxic, eco-friendly biological material namely, banana peel extract (BPE). The colorless reaction mixture turned brown and displayed UV-visible spectra characteristic of silver nanoparticles. SEM observations revealed the predominance of silver nanosized crystallites after short incubation periods, some micro-aggregates were also observed. EDS studies and XRD analysis confirmed the presence of silver nanoparticles. FTIR indicated the role of different functional groups (carboxyl, amine and hydroxyl) in the synthetic process. These silver nanoparticles displayed antimicrobial activity against fungal as well as bacterial cultures.

OBJECTIVES

In the present study, Biosynthesis of silver nanoparticles using waste fruit peel as an antimicrobial drug agent. Following objectives will be performed in the present research work:

1. Isolation and Purification of pathogenic microbes (*Staphylococcus aureus, Bacillus fusiformis, Enterobacter aerogenes, Candida albicans, Aspergillus fumigatus* and *Microsporum fulvum*).
2. Collection of waste fruits peel (*Citrus limetta* Risso., *Punica granatum* L. and *Pisum sativum* L.) and powder preparation of it.
3. Biosynthesis of silver nanoparticles from waste fruits peel.
4. Screening of waste fruits peel for the production of silver nanoparticles.
5. Characterization of silver nanoparticles (UV-Vis, XRD, SEM and FTIR).
6. Silver nanoparticles as an anti microbial agent against human pathogenic microbes.
7. Effect of biosynthesized silver nanoparticles with combination of antibiotics against human pathogenic microbes.

METHODOLOGY

1) Isolation and purification of microbes-:

The human pathogenic microbes will be obtained from Microbiology Lab, Department of Botany (D.E.I.), for bacterial cultures (*Staphylococcus aureus*, *Bacillus fusiformis* and *Enterobacter aerogenes*) NAM medium will be used whereas for fungal cultures (*Candida albicans*, *Aspergillus fumigatus* and *Microsporum fulvum*) SDA medium will be used. The petridishes will be incubated at 27-28^{o}C for two to three days. When microbial colonies appeared, it would be transferred to other dishes for the purification. Sub culturing of respective colonies till pure culture is obtained would carry out by the purification.

The culture medium used in the case of fungi and bacteria is **Sabouraud's Dextrose Agar (SDA) medium** and **Nutrient Agar Medium (NAM)** respectively. The chemical constituent of SDA medium: 40 g dextrose, 20 g peptone, 20 g agar in 1000 ml distil water. Chemical constituent of NAM medium: 5 g peptone, 3 g beef extract, 5 g sodium chloride, 20 g agar in a liter solution.

2) Collection of waste fruits peel and powder preparation-:

The waste fruits peel of *Citrus limetta*, *Punica granatum* and *Pisum sativum* will be collected from Dayalbagh Market. Then the fruits peel will be washed and boiled in distilled water for 10 min at 90^{o}C. Fruits peel (100 g) will be crushed in 200 ml of distilled water and the extract formed will be filtered through a muslin cloth. Then the filtrate will be treated with equal volumes of chilled ethanol and the resultant precipitate will be lyophilized into a powder and will be used for further experiments.

3) Biosynthesis of silver nanoparticles-:

For all experiments, the source of silver will be silver nitrate ($AgNO_3$) in distilled water. Typical reaction mixtures contained 10 mg of fruit peel extract powder in 2 ml silver nitrate solution (1mM). Other reaction conditions included incubation at 80^{o}C in a water bath for 3 min. The effect of pH on nanoparticles synthesis will be determined by adjusting the pH of the reaction mixtures (10 mg BPE, 1.0 mM silver nitrate) to 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 or 5.0. To study the effect of temperature on nanoparticles synthesis, reaction mixtures containing 10 mg fruits peel extract, and 1.0mM $AgNO_3$ at pH 3 will be incubated at 40, 60, 80 or 100^{o}C.

4) Screening of waste fruits peel (any two) for production of Silver nanoparticles-:

Screening procedure will be done on the basis of antimicrobial activity. In this, we will be measuring the activity of biologically synthesis silver nanoparticles of best two fruits peel which show maximum inhibition against one fungal species (*Candida albicans*) and one bacterial species (*Bacillus fusiformis*).

5) Characterization of silver nanoparticles-:

The silver nanoparticles synthesized after an incubation period will be characterized with the help of **UV-Vis spectrophotometer**.

Scanning electron microscopy (SEM) and elemental analysis will be performed by fabricating a drop of suspension onto a clean electric stubs and allowing water too completely evaporate. JEOL-5800-LV

SEM was used with an accelerating voltage of 15 KW and a sample current of 41μA. The samples were sputter coated with gold.

The **X-Ray diffraction (XRD)** pattern will be measured by dried powder of silver nanoparticles prepared in sample by an X'Pert Pro X-Ray diffractophotometer operated at a voltage of 40 kV and a current of 30mA with Cu Kα radiation in the range of 20^o - 80^o.

In order to determine the functional groups on the fruits peel extract powder surface and their possible involvement in the synthesis of silver nanoparticles, **Fourier Transform Infra Red (FTIR)** analysis will be carried out of the control samples (Fruit peel extract before reaction with $AgNO_3$) and the test samples (Fruit peel extract after reaction with silver salt). Model-Perkin spectrophotometer FTIR spectrum MRX-1 in the range 4000-400 cm^{-1} at a resolution of 4.0 cm^{-1} will be used. Thin sample disc will be prepared by pressing with the disc preparing machine and will be placed in FTIR for the analysis of the nanoparticles.

6) Silver nanoparticles as anti-microbial agent-:

The human pathogenic strains of *Candida albicans*, *Aspergillus fumigatus* and *Microsporum fulvum* will be used to determine the antifungal activity of the silver nanoparticles. The bacterial test cultures included *Staphylococcus aureus*, *Bacillus fusiformis* and *Enterobacter aerogenes*. The experiments on the antimicrobial activity will be carried out by three different method, **Agar Well Diffusion method** (Shanmuga *et al.,* 2002), **Colony Forming Unit method** (Sondi and Sondi, 2004) and **Biomass method** (Kunert, 1972).

Percentage inhibition will be calculated using formula:

Percentage inhibition = 100* (C-T)/C

Where

C= Fungal mycelia biomass / dry weight control.

T= Fungal Mycelia biomass / dry weight in various test concentration.

8. Effect of biosynthesized silver nanoparticles with combination of antibiotics against human pathogenic microbes -:

Combination of silver nanoparticles with low concentration of antibiotics against human pathogenic microbes *(Candida albicans*, *Aspergillus fumigatus* and *Microsporum fulvum, Staphylococcus aureus*, *Bacillus fusiformis* and *Enterobacter aerogenes).* The antimicrobial activity will be carried out by three different method, **Agar Well Diffusion method** (Shanmuga *et al.,* 2002), **Colony Forming Unit method** (Sondi and Sondi, 2004) and **Biomass method** (Kunert, 1972).

SIGNIFICANCE

There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy, and medicine. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable.

Engineered nanoparticles are rapidly becoming a part of our daily life in the form of cosmetics, food packaging, drug delivery system and therapeutic etc. Silver nitrate is highly effective on the activity of microbes. Silver based compounds are highly toxic to microorganisms. Thus, in the present study an attempt will be made to produce nano drug synthesized from novel waste fruits peel.

The bacteria and fungi that cause the human infectious disease as candidiasis, food poisonous, ring worm, aspergillosis and other dermatophytes etc. So, the outcome of this study will be focus on the exploitation of waste fruits peel for the synthesis of silver nanoparticles, to cure health impact diseases caused by bacteria and fungi.

Silver nanoparticles have several characteristics that make it currently among the most widely used nanoparticle in science. One highly useful characteristic is its antimicrobial property. Silver has long been recognized as having an inhibitory effect towards many bacterial strains and microorganisms commonly present in medical and industrial processes. If silver is transformed into a nanoparticle, this anti-microbial property is intensified, making it useful in effectively eliminating microbes.

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